REMARKS

Claims 1-98 are pending in the application. Claims 2-7, 11-13, 15-18, 21-25, 27-33, 35-36, 38-63, 66, and 68-98 have been withdrawn by the Examiner in the Office Action of July 8, 2003 as being directed to non-elected inventions. Claims 1, 8, 9, 10, 14, 19, 20, 26, 34, 37, 54, 65, and 67 have been deleted and new claims 99-112 are presented for consideration.

Rejection of claims under USC 101

Regarding the rejections of claims under USC 35 § 101, the Examiner states:

"Claim Rejections – 35 USC § 101

35 U.S.C. § 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvements thereof, may obtain a patent thereof, subject to the conditions and requirements of this title."

4. Claims 1,8,9,10,14,19,and 20 are rejected under 35U.S.C.101 because the claimed invention is directed to non- statutory subject matter. The aforementioned claims are directed to "a gene", "DNA", and "a protein". All three are products of nature and therefore are non-statutory subject matter. This rejection may be obviated by amending the claims to read "An isolated gene", Substantially purified DNA", and "An isolated protein" or similar amendments to demonstrate the "hand of man" and thus to differentiate the claimed subject matter from naturally occurring products."

Following the Examiner's suggestions, we amend claims 1, 8, 9, 10, 14, 19 and 20. We assume that the reasons of rejection under USC 35, § 101 are resolved.

2. Rejections under of claims USC 35, §102 (b)

Claims 1 and 9:

Regarding the rejections of claims 1 and 9 under USC 35 § 102 (b), the Examiner states:

"32. Claims 1 and 9 are rejected under 35 USC 102(b) as being anticipated by Slotkin et al. (August 1994) "Overexpression of the High Affinity Choline Transporter in Cortical Regions Affected by Alzheimer's Disease." J. Clin. Invest. 94(2): 696-702. Slotkin et al. teaches the isolation of brain tissue from control and Alzheimer's patients following death and the assaying of said tissue for activity of a high affinity choline transporter in neurons (Figure 1). Slotkin et al. teaches that the cholingeric neurons in Alzheimer's patients show an increased expression of a high affinity choline transporter thus meeting the limitations of claim 1 (pp. 700-701). Further, the cholingeric neurons in Alzheimer's patients showing an increased expression of a high affinity choline transporter by default express a part (at least one bp) and may well express the whole of SEQ ID NO: 5 thus meeting the limitations of claim 9 (pp.701)."

It seems that the Examiner misunderstands the relationship between rejected claims 1 and 9, and Slotkin et al., a reference cited by the Examiner. As stated by the Examiner, Slotkin et al. discloses the isolation of brain tissue from control and Alzheimer's patients following death and the assaying of said tissue for activity of a high affinity choline transporter in neurons, and further teaches that the cholingeric neurons in Alzheimer's patients show an increased expression of a high affinity choline transporter. However, claim 1 of the present application is amended as "A <u>isolated human-derived</u> gene in a cholinergic neuron which encodes a protein having high-affinity choline transporter activity" and claim 9 is amended as "<u>Substantially Purified and human-derived</u> DNA comprising a base sequence represented by Seq. ID No. 5 or its complementary". The amended claims are directed to DNA comprising human-derived gene or specific base sequence. On the other hand, Slotkin et al., cited by the Examiner, does not describe anything about the gene or DNA. In fact, as Slotkin et al. states "A direct proof of choline transporter overexpression in regions undergoing degeneration associated with Alzheimer's disease will have to await the cloning of the gene coding for the transporter and development of the corresponding cDNA probe" (p.701, last sentence of the second paragraph); there are no disclosures regarding the gene or DNA in the reference. Therefore, claims 1 and 9 cannot be rejected under 35 USC, § 102 (b) by Slotkin et al.

Further, as to the Examiner's statement that "the cholingeric neurons in Alzheimer's patients showing an increased expression of a high affinity choline transporter by default express a part (at least one bp) and may well express the whole of SEQ ID NO: 5 thus meeting the limitations of claim 9 (pp.701)", claim 9 is amended as " Substantially Purified and human-derived DNA comprising a base sequence represented by Seq. ID No. 5 or its complementary".
It is respectfully submitted that the reasons of rejection of claims 1 and 9 under USC 102 (b) are overcome.

Claims 9, 14, 20, 34 and 37:
Regarding the rejections of claims 9, 14, 20, 34 and 37 under USC 35 § 102 (b), the Examiner states:

"33. Claims 9, 14, 20, 34 and 37 are rejected under 35 USC 102(b) as being anticipated by Nikawa et al. (15 September 1990) "Primary Structure of the Yeast Choline Transporter Gene and Regulations of its Expression." The Journal of Biological Chemistry 265(26): 15996-16003 (IDS#A16). Nikawa et al. teaches a choline transporter gene and the accompanying amino acid sequence, thus meeting the limitations of claims 9, 14, and 20 (Figures 4 and 7; Table 1). Nikawa et al. teaches yeast cells expressing a choline transporter and showing choline transporter activity, thus meeting the limitations of claims 34 and 37 (Figure 9 and 10)."

For the Examiner's ease of reference, claims 9, 14 and 20 (amended as claims 102, 104 and 106, respectively) are reproduced below:

102. (amended claim based on claim 9)
Substantially Purified and human-derived DNA comprising a base sequence represented by Seq. ID No. 5 or its complementary.
104. (amended claim based on claim 14)
104. A human derived recombinant protein expressed in a cholinergic neuron and having the activity of a high-affinity choline transporter.
105. (amended claim based on claim 19)
105. A substantially-purified and human-derived protein comprising a base sequence

represented by Seq. ID No. 5.

The Examiner states that Nikawa et al. discloses the base sequence represented in Seq. ID No. 5 or amino acid sequence represented in Seq. ID No. 6. Sequences represented in Seq. ID No. 5 and 6 are derived from human, while Nilawa et al. only discloses a gene and amino acid of a yeast choline transporter. Therefore, claims 9, 14 and 20 (amended as claims 102, 104 and 106) cannot be rejected under USC 35, § 102(b) by Nikawa et al.

Further, the accession number of the nucleotide sequence disclosed in Nikawa et al. is J05603 (noted at the bottom of p.15996). The sequence does not show homology with the one represented in Seq. ID No. 5 of the present application. We also conducted homology search using the sequence represented in Seq. ID No. 5, but it does not show remarkable homology with J05603. Since the number of pages of the search results are immense, we cannot attach the results to this response, but the procedure is quite simple, and it is kindly requested that the Examiner verify this.

Therefore, there are no relationships between the sequence represented in Seq. ID No. 5 and the one disclosed in Nikawa et al. (J05603), and claims 9, 14, 20 cannot be rejected under § 102(b).

For the Examiner's ease of reference, claims 34 and 37 (amended as claims 108 and 109, respectively) are reproduced below:

108. (amended claim based on claim 34)
108. A host cell containing an expression system which can express a human-derived protein having high-affinity choline transporter activity.
109. (amended claim based on claim 37)
109. The host cell according to claim 108 ~~34~~, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 105 or 106 ~~19 or 20~~.

As noted such as "[t]ime course of repression and derepression of choline transporter" and

"[t]o an exponentially growing wild-type, (~) to a concentration of 20ug/ml.", Fig. 9 of Nikawa et al. discloses a cell having high-affinity choline transporter activity (p. 16000-16001). However, the choline transporter used in the reference is yeast, and there are no disclosures regarding a human-derived choline transporter. Therefore, we assume that claim 37 after amendment (i.e., claim 109) should not be rejected under USC 35 § 102(b) by the reference.

Further, claim 34 was amended to include a limitation of "a high-affinity choline transporter in a cholinergic neuron", as made to claim 1 in the Response to the Restriction Requirement f;ed February 27, 2003.

For the reasons mentioned above, we assume that the reasons of rejection to claims 9, 14, 20, 34, 37 under USC 35, § 102 (b) are resolved.

Claims 14:

Regarding the rejections of claims 14 under USC 35 § 102 (b), the Examiner states:

"34. Claim 14 is rejected under 35 USC 102(b) as being anticipated by Knipper et al. (18 June 1991) "Purification and reconstitution of the high affinity choline transporter." Biochiemica et Biophysica Acta, 1065(2): 107-113. Knipper et al. teaches the purification and functional characterization of a protein with high affinity choline transporter activity, thus meeting the limitations of claim 14 (Figure 5 and 6; pp.113)."

Claim 14 of the present invention is amended as "A substantially-purified and human-derived protein having the activity of a high affinity choline transporter that can be expressed in the cholinergic neurons", and there are no disclosures regarding "a human-derived recombinant protein having high-affinity choline transporter" in Knipper et al.

As explained above, we respectfully submit that the reasons of rejection under USC 102(b) are resolved.

3. Rejections of claims under USC 35, §112, 1st paragraph

Regarding the rejections of claims under USC 35 § 112 1st paragraph, the Examiner states:

"The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out any distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1, 8, 9, 10, 14, 20, 26, 34,and 37 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for an isolated gene comprising an amino acid sequence represented by *SEQ ID NO: 6* or a host cell transfected with same and a protein or fusion protein comprising by *SEQ ID NO: 6*, does not reasonably provide enablement for a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by *SEQ ID NO: 6* or protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by *SEQ ID NO: 6*. The specification does not enable any person skilled in the art to which it pertains, or with which it most nearly connected, to make the invention commemsurate in scope with these claims.

6. Regarding derivatives and fragments of *SEQ ID NO: 6*, the problem of predicting protein structure from sequence data and in turn utilizing predicted structural determinations to ascertain functional aspects of the protein is extremely complex. While it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited. Certain positions in the sequence are critical to the protein's structure/function relationship, e.g. such as various sites or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites. These or other regions may also be critical determinants of antigenicity. These regions can tolerate only relatively conservative substitutions or no substitutions [see Wells (18 September 1990)" Additivity of Mutational Effects in Proteins." Biochemistry 29 (37) : 8509-8517; Ngo *et al.* (2 March 1995) "The Protein Folding Problem and Tertiary Structure Prediction, Chapter 14:

Computational Complexity Protein Structure Prediction, and the Levinthal Paradox" pp.492-495]

7. For instance, Okuda et al. (22 November 2002) "Single nucleotide polymorphism of the human high affinity choline transporter rate." The Journal of Biological Chemistry, 277 (47): 45315-45322 teaches that the presence of a single nucleotide polymorphism in the human CHT1 polymorphism in the human CHT1 gene (189V) results in a 40-50% decrease in V(max) for choline uptake rate compared with the wild type. However, there was no alteration in the apparent affinities for choline, sodium, chloride, and the specific inhibitor hemicholinum-3 (Figures 3,4, and 6). Thus a single amino acid change can significantly reduce the activity of a high-affinity choline transporter.

8. In this regard, Applicant has provided little or no guidance beyond the mere presentation of sequence data to enable one of ordinary skill in the art to determine, without undue experimentation, the positions in the protein which are tolerant to change (e.g. such as by amino acid substitutions or deletions), and the nature and extent of changes that can be made in these positions. Although the specification outlines art-recognized procedures for producing and screening for active muteins, this is not adequate guidance as to the nature of active derivatives that may be constructed, but is merely an invitation to the artisan to use the current invention as a starting point for further experimentation. Even if an active or binding site were identified in the specification, they may not be sufficient, as the ordinary artisan would immediately recognize that an active or binding site must assume the proper three-dimensional configuration to be active, which conformation is dependent upon surrounding residues; therefore substitution of non-essential residues can often destroy activity. The art recognizes that function cannot be predicted from structure alone [Bork (2000) "powers and Pitfalls in Sequence Analysis: The 70% Hurdle." Genome Research 10:398-400; Skolnick and Fetrow (2000) "From gene to protein structure and function: novel applications of computational approaches in the genomic era." Trends in Biotech. 18(1):34-39, especially p. 36 at Box 2; Doerks et al., (June 1998) "Protein annotation: detective work for function prediction." Trends in Genetics 14(6): 248-250; Smith and Zhang (November 1997) "The Challenges of genome sequence annotation or 'The devil is in the details'" Nature Biotechnology 15: 1222-1223; Brenner (April 1999) "Errors in genome annotation." Trends in Genetics 15(4):132-133; Bork and Bairoch (October 1996) "Go hunting

in sequence databases but watch out for the traps." Trends in Genetics 12(10): 425-427]. Due to the large quantity of experimentation necessary to generate the infinite number of derivatives recited in the claims and possibly screen same for activity, the lack of direction/guidance presented in the specification regarding which structural features are required in order to provide activity. The absence of working examples directed to same, the complex nature of the invention, the state of the prior art which establishes the unpredictability of the effects of mutation on protein structure and function, and the breadth of the claims which fail to recite any structural or functional limitations, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope."

We respond to the examiner's suggestions as follows:

To summarize the Examiner's statements, the specification of the present application enables for an isolated gene comprising an amino acid sequence represented by SEQ ID NO: 6 or a host cell transfected with same and a protein or fusion protein comprising by SEQ ID NO:, but does not reasonably provide enablement for a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by SEQ ID NO:6 or protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by SEQ ID NO:6, and therefore claims 1, 8, 9, 10, 14, 20, 26, 34 and 37 of the present invention are rejected under 35 USC 112, first paragraph.

First, it is respectfully submitted that claims 8, 20, 26 and 37 are directed to the protein represented by SEQ ID NO:6, and claims 9, 10, 34 are directed to the DNA represented by SEQ ID NO: 5, but claims 1 and 14 are directed neither to the protein represented by SEQ ID NO:6 nor the DNA represented by SEQ ID NO: 5. From the statements made by the Examiner, the statement regarding the protein represented by SEQ ID NO:6 or the DNA represented by SEQ ID NO: 5, claims 1 and 14 cannot be rejected for the reason stated above.

As to the Examiner's statement regarding "a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by SEQ ID NO:6", it should exactly be "a protein comprising an amino acid

sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by SEQ ID NO: 6, and having high-affinity choline transporter activity". The claims does not encompass all the proteins "comprising an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence represented by SEQ ID NO:6", but rather it is limited to a protein "having high-affinity choline transporter activity". The Examiner seems to look over this point, and reconsideration of this point is kindly requested.

As stated by the Examiner, " it is known that many amino acid substitutions are generally possible in any given protein", and it cannot be concluded that it would require undue experimentation for people skilled in the art to carry out a claimed invention using a protein comprising amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence, or a gene encoding the protein. Within a specific sequence of amino acids (for example, the amino acid sequence represented by SEQ ID NO:6), people skilled in the art can arbitrarily use an amino acid sequence where one or a few amino acids are deficient, substituted, or added in the amino acid sequence, and the experimentation can be carried out following the same procedures used for the amino acid sequence without deficiency, substitution or addition. In this sense, it cannot be said that such amino acid sequences require undue experimentation. Therefore, it is not valid to conclude that the claims are rejected under 35 USC 112, paragraph 1 (enablement). The same applies to the base sequence encoding the proteins.

The Examiner states that "Certain positions in the sequence are critical to the protein's structure/function relationship", and "the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited", but as stated by the Examiner, "many amino acid substitutions are generally possible in any given protein" and if such amino acid sequences with substitution, deficiency or addition can also produce the same effect in the same manner as the case of amino acid sequence without substitution, deficiency or addition, it should be within the scope of the invention as a technical idea. There is also no undue experimentation. Is the case where there are unexpected and useful effect, differing from the case of using amino acid sequence without substitution, deficiency or

addition, the novel useful effect itself should be patented independently because it is within the scope of a novel technical idea, i.e., invention. As the Examiner acknowledges, in the case where such a novel and useful activity of a protein is found, it would be a new technical idea independent from the present invention, but it would be inadequate to reject the claims of the present application using such a concern that there might exist such an "invention" that has not been found so far. It is respectfully submitted that such a rejection would not be supported by any section of 35 USC.

Using Okuda et al. (22 November 2002) as an example, the Examiner states that " a single amino acid change can significantly reduce the activity of a high-affinity choline transporter". As stated above, however, the claims are restricted to "high-affinity choline transporter". Further, Okuda et al. is published on November 22, 2002, and the international filing date of this application is August 18, 2000. This is tantamount to rejecting the claimed invention of the present application using a publication published after the filing date of this application It is evident that such a rejection is groundless.

More importantly, if a claim such as "a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6" is not patentable, and only a claim directed to " a protein comprising an amino acid sequence represented by Seq. ID No. 6" is patentable, since "it is known that many amino acid substitutions are generally possible in any given protein, the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited" as stated by the Examiner, the patent can easily be avoided by using such variations. Such a situation is clearly disadvantageous for applicants, and they might consider whether it is really valuable to obtain a patent rights in the United States with a large amount of money. Reconsideration is respectfully requested for a proper practice of the patent application in the US.

By the way, it was about 15 years ago that the USPTO requested the Japan Patent Office to allow claims with scope as wide as possible if there are no prior art references. The Japan Patent Office made clear as part of the Examination Standard that the JPO allows a claim such as "An

isolated gene which encodes a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having high-affinity choline transporter activity., and it is kindly requested that the same standard be applied to the present case.

With the above explanations, we assume that the reasons of rejection under 1st paragraph under 35 USC § 112 are resolved. However, if the Examiner considers that more explanations are necessary, we kindly ask the Examiner to notify us of it.

Regarding the rejections of claims under 35 USC § 112 1st paragraph, the Examiner states:

" The claims are drawn to gene encoding polypeptides and polypeptides having high-affinity choline transporter activity. The claims do not require that the polypeptides possess any particular conserved structure. Thus, the claims are drawn to a genus polypeptides that is defined by broad activity.

To provide adequate written description and evidence of possession of a claimed genus, the factor to be considered includes disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, and any combination thereof. In this case, the only factor present in the claim that is sufficiently disclosed is a relatively defined function. The specification does not identify any particular portion of the structure that must be conserved, nor does or provide a disclosure of structure/function correlation. The distinguishing characteristics of the claimed genus are not described. The only adequately described species is polypeptode comprising *SEQ ID NO: 6*. No active variants are disclosed. Accordingly, the specification does not provide adequate written description of the claimed genus.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the *invention.* The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See *Vas-Cath* at page 116). As discussed above, the skilled artisan

cannot envision the detailed chemical structure of the encompassed genus of polypeptides, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The compound itself is required. See *Fiers v. Revel*, 25 USPQ2d 1601 at 1606(CAFC 1993) and *Amgen Inc. v.Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

One cannot describe what one has not conceived. *See Fiddes v. Baird,* 30 USPQ2d 1481 at 1483. In *Fiddes*, Claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, only isolated polypeptides comprising the amino acid sequence set forth in *SEQ ID NO: 6,* but not the full breadth of the claim meets the written description provision of 35 U.S.C. § 112, first paragraph. Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C.§112 is severable from its enablement provision.

Claims 64, 65 and 67 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for an *in vitro preparing method of a cell having high-affinity choline transport activity characterized in introducing DNA encoding of SEQ ID NO:5, into said cell and the cell made my said in vitro method,* does not reasonably provide enablement for the practicing of said method *in vivo* (i.e. in patients) or transgenic animals made by the claimed method. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate it scope with there claims."

We respond to the Examiner's suggestions as follows:
The Examiner states that claims 1, 8, 9, 10, 14, 20, 26, 34 and 37 of the present invention are directed to a polypeptide having high-affinity choline transporter activity and a gene encoding the polypeptide, yet the claims do not describe the preserved structures, the gene of the claimed polypeptides is indefinite, and the claims do not satisfy the description requirement. As a disclosure of a gene, the Examiner states that "[t]o provide adequate written description and

evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus" and "[t]o provide adequate written description and evidence of possession of a claimed genus, the factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, and any combination thereof." In other words, the Examiner states that the claims of the present invention do not define a claimed genus (in this case, a protein having a high-affinity choline transporter activity) defined by factors to specify the genus, including "disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, and any combination thereof".

Relating to this point, the claims are defined by a functional characteristic of " having a high-affinity choline transporter activity" as a factor to define the genus, and the functional characteristic is included in the factors to define a genus. As suggested by the Examiner, there are no requirements to satisfy all of the factors to define a genus. We kindly ask the Examiner to recall that the claims of the present invention are defined by a factor as long as it [the factor?] can clearly distinguish the genus from others.

Further, the specification of the present invention includes the following description, which is a standard for identifying the choline intake activity:

Best Mode for Carrying out the Invention

The cDNA of nematode high-affinity choline transporter of the present invention, being described in Seq. ID No. 1, can be obtained by injecting each cRNA prepared from candidate full-length cDNAs, which are expected as a member of Na^+-dependent transporter family according to C. elegans genome project, into oocytes of Xenopus, and examining the uptake of choline. The high-affinity uptake of choline in brain synaptosomes of mammals was completely inhibited by 1 μMhemicholinium-3 (HC3) (Ki=10-100 nM), while the low-affinity uptake of choline, which is distributed in every cells, was inhibited only by HC3 with higher concentration (Ki=50 μM). Therefore, the sensitivity to 1 μM HC3 can be used as criteria of high-affinity

choline uptake during the process. For example, it is possible to confirm the identification, the expression, and the localization of an object gene from the candidate cDNA of a nematode (C. elegans) as follows." (emphasis added)

From the above explanations, it is concluded that the Examiner's statement that claims 1, 8, 9, 10, 14, 20, 26, 34 and 37 are rejected under 35 USC § 112, 1st paragraph does not hold. We assume that the above explanations are enough to overcome the reasons of rejection, but if the Examiner considers that more explanation would be necessary, please kindly ask the Examiner to let us know. We would be glad to answer to the Examiner's questions or concerns.

Claims 64, 65 and 67:

Regarding the rejections of claims 64, 65 and 67 under USC 35 § 112 (b), the Examiner states:

"15. Claims 64, 65 and 67 are rejected under 35 U.S.C.112, first paragraph, because the specification, while being enabling for an *in vitro preparing method of a cell having high-affinity choline transport activity characterized in introducing DNA encoding of SEQ ID NO:5, into said cell and the cell made my said in vitro method,* does not reasonably provide enablement for the practicing of said method *in vivo* (i.e. in patients) or transgenic animals made by the claimed method. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with the claims.

16. The above invention is drawn to methods of transfecting a cell with a DNA encoding a protein with high-affinity choline transporter activity. The language of said claims encompasses both *in vivo* and *in vitro* transfection (DNA introduction). The specification teaches that DNA encoding a protein with high-affinity choline transporter activity can be successfully introduced into oocytes and COS7 cells.

17. But the claims are drawn very broadly to methods of introducing DNA encoding a protein with high-affinity choline transporter activity into cell lines and patients (i.e. gene therapy). Since the specification fails to provide any guidance for the successful introduction of a DNA

encoding a protein with high-affinity choline transporter activity into a patient (whether human or animal) and since resolution of the various complications in regards to targeting a particular gene in an organism is highly unpredictable, one of skill the art would have been unable to practice the invention without engaging in undue trial and error experimentation.

18. The specification as filed does not provide any guidance or examples that would enable a skilled artisan to use the disclosed methods of introducing a DNA encoding a protein with high-affinity choline transporter activity into animal. Additionally, a person skilled in the art would recognize that predicting the efficacy of using a specific DNA encoding a protein with high-affinity choline transporter activity *in vivo* based solely on its performance *in vitro* is highly problematic. Thus, although the specification prophetically considers and discloses general methodologies of using the claimed methods in *in vivo* gene therapy or to make transgenic animals, such a disclosure would not be considered enabling since the state of gene therapy is highly unpredictable [see Verma and Somia (18 Septe mber 1997) "Gene therapy-promises, problems and prospects." Nature 389;239-241;Kanda (September 2001) "Gene Therapy: A Battle Against Biological Barriers" Current Molecular Medicine 1(4);493-499; Eck and Wilson (1996) Chapter 5; "Gene-Based Therapy" Goodman & Gilmans's The Pharmacological Basis Of Therapeutics 9th Ed. (pp.77-100)] The factors listed below have been considered in the analysis of enablement:

(A) The breadth of the claims;

(B) The nature of the invention;

(C) The state of the prior art;

(D) The level of one of ordinary skill;

(E) The level of predictability in the art;

(F) The amount of direction provided by the inventor;

(G) The existence of working examples; and

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure

19. The following references are cited herein to illustrate the state of the art of a gene encoding a protein with high-affinity choline transporter activity.

20. Regarding derivatives and fragments of DNA encoding *SEQ ID NO:6,* the problem of predicting protein structure from sequence data and in turn utilizing predicted structural determinations to ascertain functional aspects of the protein is extremely complex. While it is known that many amino acid substitutions are generally possible in any given protein, the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited. Certain positions in the sequence are critical to the protein's structure/function relationship, e.g. such as various sites or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites. These or other regions may also be critical determinations of antigenicity. These regions can tolerate only relatively conservative substitutions or no substitutions [see Wells (18 September 1990) "Additivity of Mutational Effects in Proteins." Biochemistry 29(37): 8509-8517; Ngo et al. (2 March 1995) "The Protein Folding Problem and Tertiary Structure Prediction, Chapter 14: Computational Complexity Protein Structure Prediction, and the Levinthal Paradox" pp.492-495]

21. For instance, Okuda et al. (22 November 2002) "Single nucleotide polymorphism of the human high affinity choline transporter rate. "The Journal of Biological Chemistry 277 (47):45315-45322 teaches that the presence of a single nucleotide polymorphism in the human CHT1 polymorphism in the human CHT1 gene (189V) results in a 40-50% decrease in V(max) for choline uptake rate compared with the wild type. However, there was no alteration in the apparent affinities for choline, sodium, chloride, and the specific inhibitor hemicholinum-3 (Figures 3,4, and 6). Thus a single amino acid change can significantly reduce the activity of a high-affinity choline transporter.

22. In this regard, Applicant has provided little or no guidance beyond the mere presentation of sequence data to enable one of ordinary skill in the art to determine, without undue experimentation, the positions in the protein which are tolerant to change (e.g. such as by amino acid substitutions or deletions), and the nature and extent of changes that can be made in these positions. Although the specification outlines art-recognized procedures for producing and screening for active muteins, this is not adequate guidance as to the nature of active derivatives

that may be constructed, but is merely an invitation to the artisan to use the current invention as a starting point for further experimentation. Even if an active or binding site were identified in the specification, they may not be sufficient, as the ordinary artisan would immediately recognize that an active or binding site must assume the proper three-dimensional configuration to be active, which conformation is dependent upon surrounding residues; therefore substitution of non-essential residues can often destroy activity. The art recognizes that function cannot be predicted from structure alone [Bork (2000) "powers and Pitfalls in Sequence Analysis: The 70% Hurdle." Genome Research 10:398-400; Skolnick and Fetrow (2000) " From gene to protein structure and function: novel applications of computational approaches in the genomic era." Trends in Biotech. 18(1):34-39, especially p.36 at Box 2; Doerks et al., (June 1998) "Protein annotation: detective work for function prediction." Trends in Genetics 14(6): 248-250; Smith and Zhang (November 1997) "The Challenges of genome sequence annotaion or 'The devil is in the details'." Nature Biotechnology 15: 1222-1223; Brenner(April 1999) "Erroes in gerome annotation." Trends in Genetics 15(4):132-133; Bork and Bairoch (October 1996) "Go hunting in sequence databases but watch out for the traps." Trends in Genetics 12(10): 425-427] Due to the large quantity of experimentation necessary to generate the infinite number of derivatives recited in the claims and possibly screen same for activity, the lack of direction/guidance presented in the specification regarding which structural features are required in order to provide activity, the absence of working example directed to same, the complex nature of the invention, the state of the prior art which establishes the unpredictability of the effects of mutation on protein structure and function, and the breadth of the claims which fail to recite any structural or functional limitations, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope.

23. In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed *in vivo* would require the *de novo* determination of conditions for successful incorporation of a DNA encoding a protein with high-affinity choline transporter activity into the genome of an animal. In the absence of any guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed. Thus the specification of the instant application fails to provide adequate guidance for

one of skill in the art to overcome the unpredictability and challenges of applying results from *in vitro* experiments to the *in vivo* transfection of a gene encoding a protein having high-affinity choline transporter activity as exemplified in the references above."

We respond to the Examiner's suggestions as follows:
To summarize the Examiner's statement cited above, the specification of the present invention "enables for an *in vitro* preparing method of a cell having high-affinity choline transport activity characterized in introducing DNA encoding of SEQ ID NO:5, into said cell and the cell made my said *in vitro* method, does not reasonably provide enablement for the practicing of said method *in vivo* (i.e. in patients) or transgenic animals made by the claimed method, and therefore claims 64, 65 and 67 are rejected under 35 USC §112 paragraph 1.

As a reason to make distinction between an *in vitro* method on one hand and an *in vivo* method or transgenic animals made by the claimed method, the Examiner takes the position that the state of gene therapy is highly unpredictable, citing a number of references. It would be appropriate to point out here that Okuda et al. (22 November 2002) is cited again as an example, but Okuda et al. does not support the Examiner's statement. Further, paragraph 21 to 22 and paragraph 7 to 8 are exactly the same in the Office Action. We assume that the reference is mistakenly suggested by the Examiner in the Office Action, and would kindly ask the Examiner to review it for confirmation.

The Examiner states that "the state of gene therapy is highly unpredictable, and claimed invention cannot be carried our for this reason. However, the Examiner does not point our any references directly showing that the claimed invention cannot be carried out. In other words, the Examiner simply states that the claimed invention **might be impossible to carry out** if the method is "an *in vivo* method or transgenic animals made by the claimed method".

As the Examiner recognizes that "enabling for an *in vitro* preparing method of a cell having high-affinity choline transport activity characterized in introducing DNA encoding of SEQ ID NO:5 into said cell and the cell made my said *in vitro* method", the preparation of a cell haivng high-affinity choline transporter activity in the present application can be carried out in principle.

Thus, following the method stated in the specifcation, an *in vivo* method or transgenic animals made by the claimed method can be carried out in principle as well. The Examiner's rejection of the claims is simply based on the concern that " an *in vivo* method or transgenic animals made by the claimed method" might be carried out, which is clearly not appropriate. Further, the Examiner does not point out any essential factors distinguishing an *in vitro* method on one hand and an *in vivo* method or transgenic animals made by the claimed method on the other. Further, there might be any distinctions between an *in vitro* method on one hand and an *in vivo* method or transgenic animals made by the claimed method on the other, there are other factors involved in an *in vivo* method or transgenic animals made by the claimed method, but not in an *in vitro* method. If the Examiner would like to draw a line between the two, we kindly request the Examiner to provide pieces of evidence to show why this distinction but not others comes into play It is kindly asked that the Examiner also provide evidence (e.g., references) and legal grounds (e.g., laws, rules or cases) supporting the view of making a distinction between an *in vitro* method on one hand and an *in vivo* method or transgenic animals made by the claimed method on the other.

We assume that the above explanations resolved the reasons of rejection of claims 64, 65 and 67 under 35 USC §112 first paragraph.

Claim 9:

Regarding the rejections of claim 9 under USC 35 § 112 2nd paragraph, the Examiner states: "24. Claim 8 is rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

25. Claim 9 recites: "SEQ ID NO: 5 or its complementary sequence and a part of whole of these sequences". It is unclear whether claim 9 is directed to a fusion or was indented to recite all in the alternative."

Claim 9 is amended as containing "[s]ubstantially Purified and human-derived DNA comprising a base sequence represented by Seq. ID No. 5 or its complementary.

We assume that the reason of rejection of claim 9 under USC 35 § 112, second paragaph is resolved.

Claims 10:

Regarding the rejections of claims 10 under 35 USC § 112 second paragraph, the Examiner states:

"26. Claim 9 recites: "SEQ ID NO: 5 or its complementary sequence and a part or whole of these sequences". It is unclear whether claim 9 is directed to a fusion or was intended to recite all in the alternative.

27. The term "stringency" in claim 10 is a relative term which renders the claim indefinite. The term "stringency" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skilled in the art would not be reasonably apprised of the scope of the invention. Neither the specification nor the art defined the term unambiguously. Thus, the metes and bounds of the claims cannot be determined."

Claim 10 states that "[s]ubstantially Purified and human-derived DNA encoding a protein that hybridize with DNA constituting the gene according to claim 102~~9~~ under stringent considerations, and have high affinity choline transporter activity" and it is clear that claim 10 is directed to substantially purified DNA.

Further, as to the wording of "stringency" which is pointed out in the Examiner, there is a statement in the specification as follows:

" Examples of a protein having high-affinity choline transporter activity of the present invention include a protein derived from natural materials and a recombinant protein. In addition to the ones represented by Seq. ID Nos. 2, 4, 6 and 8, which are specifically disclosed above, a protein comprising an amino acid sequence wherein one or a few amino acids are deficient, substituted or added in amino acid sequences represented by Seq. ID Nos. 2, 4, 6 and 8, and having high-affinity choline transporter activity is also included. These proteins can be prepared by known methods. Further, examples of a gene or DNA encoding a protein having high-affinity choline

transporter activity of the present invention include, in addition to the ones represented by Seq. ID Nos. 1, 3, 5 and 7, which are specifically disclosed above, a gene or DNA which encodes a protein comprising an amino acid sequence wherein one or a few amino acids are deficient, substituted or added in amino acid sequences represented by Seq. ID Nos. 2, 4, 6 and 8, and having high-affinity choline transporter activity, and DNA which encodes a protein hybridizing with said gene or DNA under a stringent condition and having high-affinity choline transporter activity. These genes and DNAs can be prepared by known methods."

As is clear from the above citation, the DNA in claim 10 can be "prepared by known methods" using the DNA of the present invention. As a known method, for example, hybridization at 42°, and rinse at 42° using buffer containing 1 ×SSC, 0.1% of SDS can be exemplified, and as a factor affecting the stringency of hybridization, there are various factors other than temperature, and a person skilled in the art knows well that the stringency means that the same level of stringency can be realized by combining various factors in various ways to the level of the stringency of hybridization mentioned above. In addition to the fact that a person skilled in the art is able to judge whether a hybridization is stringent or not, a number of statements are made about hybridization in various references, and we assume that it does not require any specific statement regarding the term.

We respectfully submit that the rejection of claims 1 and 9 under USC 35, 102 (b) has been overcome.

Claims 20, 26 and 37:

Regarding the rejections of claims 20, 26 and 37 under 35 USC § 112 second paragraph, the Examiner states:

"Claims 20, 26, and 37 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

"Claims 20, 26, and 37 refer to "human high-affinity choline transporter activity". It is not clear from the instant specification or the prior art as to what the meters and bounds of "human high-

affinity choline transporter activity" are.

Regarding "human high-affinity choline transporter activity" in the specification of the present invention, there is a description as follows:

"The cDNA of human high-affinity choline transporter of the present invention, being represented by Seq. ID No.5, can be prepared, for example, as follows: data base search was conducted with the amino acid sequence of nematode (C. elegans) CHO-1 to find a sequence of specific human genome DNA fragment having significant homology (R-107P12, a clone of human genomic survey sequence; GenBank accession number: AQ316435); a gene-specific primers for PCR were designed based on a base sequence of said DNA fragment; 5'-RACE (rapid amplification of cDNA ends) and 3'-RACE were conducted using Marathon-Ready™ cDNA (Clontech) of human whole brain, together with an attached adapter primer; the obtained PCR product was cloned into a cloning vector for PCR, and a base sequence of inserted DNA was determined. In addition, an amino acid sequence expected from this DNA sequence is represented by Seq. ID No. 6. A protein having human high-affinity choline transporter activity represented by said Seq. ID No. 6 can be constructed by a usual method on the basis of DNA sequence information shown in Seq. ID No. 5." (underline added)

From the above citation, it is clear that "human high-affinity choline transporter" means human-derived high-affinity choline transporter. Although the Examiner raises a question regarding "human high-affinity choline transporter activity", it is clear that it means "activity that a human high-affinity choline transporter has".

We assume that the above explanation clearly answers the questions raised by the Examiner, but to make clearer the meaning of the term "human high-affinity choline transporter", it is amended as "isolated and human-derived protein having a human high-affinity choline transporter".

We assume that the reasons of rejection of claims 20, 26 and 37 under USC 35, 112, second paragraph are resolved.

Claims 26 and 37:

Regarding the rejections of claims 20, 26 under 35 USC § 112 second paragraph, the Examiner states:

"30. Claims 26 and 37 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particular point out and distinctly claim the subject matter which applicant regards as the invention.
31. Claims 26 and 37 refer to high-affinity choline transporter or claim 20 but claim 20 does not provide further details of such activity."

Claim 20 is amended as follows:

"106. A substantially-purified and human-derived protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having human high-affinity choline transporter activity."

As a reply to the Examiner's statement, we would like to point out the fact that "high-affinity choline transporter" is a term normally used by the people skilled in the art, and does not require any special explanation to understand it. The fact is also clear from the titles of the references cited by the Examiner, because the titles involves the term "high-affinity choline transporter". In fact, Slotkin *et al.* (August 1994) "Overexpression of the High Affinity Choline Transporter in Cortical Regions Affected by Alzheimer's Disease." J. Clin. Invest. **94** (2): 696-702, Knipper *et al* (18 June 1991) "Purification and reconstitution of the high affinity choline transporter." Biochimica et Biophysica Acta. **1065 (2)**: 107-113, Okuda *et al.* (22 November 2002) "Single nucleotide polymorphism of the human high affinity choline transporter alters transport rate." The Journal of Biological Chemistry **277** (47): 45315-45322, use the term "high-affinity choline transporter" as part of their titles. Therefore, the term "high affinity choline transporter" is not the one that requires a special explanation or definition.
We assume that the reasons of rejection of claims 26 and 37 under 35 USC § 112, second paragraph are resolved.

In summary, claims 1, 8, 9, 10, 14, 19, 20, 26, 34, 37, 54, 65, and 67 have been deleted and new claims 99-102 are now under consideration. Having addressed all of the Examiner's concerns, Applicants believe the application is in condition for allowance.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

It is not believed that any petition for extension of time or fees are due with the filing of this amendment. Should any fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 22-0261 for any such fees; and applicants hereby petition for any needed extension of time

Respectfully submitted,

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